AMERICAN JOURNAL OF PHYSICAL ANTHROPOLOGY 110:129–142 (1999)

Complex, Compound Inversion/Translocation Polymorphism in an Ape: Presumptive Intermediate Stage in the Karyotypic Evolution of the Agile Gibbon *Hylobates agilis*

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KEY WORDS cytogenetics; taxonomy; captive management; Hylobatidae; reciprocal translocation; meiosis

ABSTRACT Karyotypic variation in five gibbon species of the subgenus *Hylobates* ($2n = 44$) was assessed in 63 animals, 23 of them wild born. Acquisition of key specimens of *Hylobates agilis* (agile gibbon), whose karyotype had been problematic due to unresolved structural polymorphisms, led to disclosure of a compound inversion/translocation polymorphism. A polymorphic region of chromosome 8 harboring two pericentric inversions, one nested within the other, was in turn bissected by one breakpoint of a reciprocal translocation. In double-inversion + translocation heterozygotes, the theoretical meiotic pairing configuration is a double inversion loop, with four arms of a translocation quadrivalent radiating from the loop. Electron-microscopic analysis of synaptonemal complex configurations consistently revealed translocation quadrivalents but no inversion loops. Rather, nonhomologous pairing was evident in the inverted region, a condition that should preclude crossing over and the subsequent production of duplication-deficiency gametes. This is corroborated by the existence of normal offspring of compound heterozygotes, indicating that fertility may not be reduced despite the topological complexity of this polymorphic system. The distribution of inversion and translocation morphs in these taxa suggests application of cytogenetics in identifying gibbon specimens and avoiding undesirable hybridization in captive breeding efforts. Am J Phys Anthropol 110:129–142, 1999.

Gibbons (genus *Hylobates,* family Hylobatidae) constitute a sister group to the great apes (Pongidae) and humans (Hominidae) (Groves, 1972). The four subgenera of gibbons recognized by classical taxonomic criteria are further characterized by four unique karyotypes and diploid numbers (Chiarelli, 1972; Markvong, 1973; Prouty et al., 1983). Comparative cytogenetic studies reveal striking degrees of genomic rearrangement since divergence of the four groups from a common ancestor (Dutrillaux et al., 1975; Myers and Shafer, 1979; Couturier et al., 1982; Prouty et al., 1983; vanTuinen and Ledbetter, 1983). Among the four karyo-

Grant sponsor: National Science Foundation; grant number: DEB-9201509.

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Received 9 December 1998; accepted 4 June 1999.

types, only 30–60% of the haploid length is cytogenetically conserved to the extent that putatively homologous chromosomes can be identified (Couturier et al., 1982; vanTuinen and Ledbetter, 1983). Gene mapping in gibbons has disclosed considerable disruption of ancient, conserved linkage groups, underscoring the great extent of genomic rearrangement (Turleau et al., 1983; vanTuinen and Ledbetter, 1986; Cochet et al., 1987; vanTuinen and Ledbetter, 1989). This contrasts markedly to comparisons of great apes and humans with Old World monkeys (Cercopithecoidea): between these more distantly related taxa all chromosomes have identifiable homologues among different taxa (Dutrillaux et al., 1978; Yunis and Prakash, 1982). Most inferences of homology based on chromosome banding have been confirmed by gene mapping (Finaz et al., 1975; Thiessen and Lalley, 1987; Creau-Goldberg et al., 1990).

Virtually all modes of chromosomal rearrangement observed in karyotypic divergence of mammals are recognized in gibbons: pericentric and paracentric inversion, Robertsonian and tandem fusion, chromosomal fission, and reciprocal-translocation (Dutrillaux et al., 1975; Couturier et al., 1982; vanTuinen and Ledbetter, 1983). It might be expected that taxa characterized by such extensive karyotypic differentiation would exhibit intermediate stages of change, i.e., structural polymorphisms. Indeed among the earliest studies conducted, pericentric-inversion heteromorphism was noted in *Hylobates muelleri* (Mueller's gibbon), one of the six species of the subgenus *Hylobates* that all possess 2n = 44 (Tantravahi et al., 1975). Inversion heteromorphism for the same chromosome pair was noted in one of two *Hylobates agilis* (agile gibbon) (vanTuinen and Ledbetter, 1983). A more comprehensive study of the six 2n = 44 species (Stanyon et al., 1987) revealed the existence of three distinct pericentric-inversion morphs involving the same chromosomal pair, with *H. muelleri, Hylobates moloch* (moloch gibbon), and *H. agilis* maintaining all three morphs.

The nature of the contrasts between the four karyotypes in gibbons led to the hypothesis that reciprocal-translocation predominated in chromosomal evolution of *Hylobates* (vanTuinen and Ledbetter, 1983). While the magnitude of genomic restructuring has rendered the interpretation of many translocation events difficult or impossible, this hypothesis was corroborated by gene mapping and in situ hybridization, which revealed novel gene linkage groups most consistent with translocation (Turleau et al., 1983; Cochet et al., 1987; vanTuinen and Ledbetter, 1989; Arnold et al., 1996). In the present study, we investigate a complex cytogenetic polymorphism that is possibly an intermediate stage in the differentiation of the karyotype of *H. agilis*.

MATERIALS AND METHODS

Specimen identification

Hylobates lar (lar gibbon) (18 specimens), *H. moloch* (7 specimens), *H. muelleri* (5 specimens), *Hylobates pileatus* (pileated gibbon) (10 specimens), *H. agilis* (18 specimens), and five interspecific hybrids were examined in this study. Specific and subspecific status was determined by comparing pelage to study skins in the Field Museum of Natural History (Chicago), the National Museum Of Natural History (Washington), the American Museum of Natural History (New York), the Museum Zoologicum Bogoriense (Bogor), and the National University of Singapore Zoological Reference Collection (Singapore). Additional criteria followed Groves (1972), Marshall and Sugardjito (1986), Geissmann (1995), and Mootnick and Sheeran (1996). Vocalizations were compared to Marshall and Marshall (1978) and Marshall and Sugardjito (1986). All specimens except *H. lar* 373, 433, 669, 823238, 864781, 896664, and *H. agilis* 521A and 588335 were observed in person or by photographs, and heard by one of us (ARM). The vocalizations of specimens 373, 433, 669, 823238, 864781, and 896664 were confirmed by sonogram (J.T. Marshall, personal communication), and specimen 3135 was observed vocalizing (D. Chivers, personal communication).

Metaphase chromosome preparation

Peripheral blood lymphocytes were cultured in RPMI 1640 + 15% fetal bovine

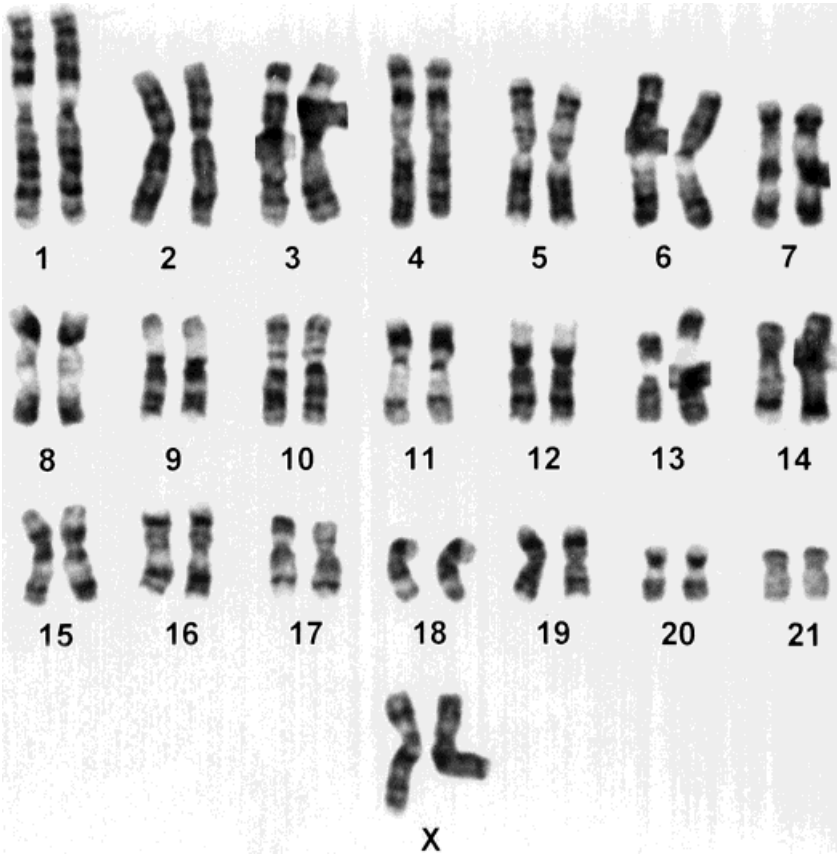

Fig. 1. G-banded karyotype of *H. moloch* HMO801 showing homozygosity for the most prevalant chromosome 8 and 9 morphs (8b), i.e., the only morphs observed in all 2n = 44 species including *H. agilis*. All other chromosome pairs are apparently identical among 2n = 44 gibbons.

serum and 2% phytohemagglutinin, and harvested using standard techniques following 20 min colcemid arrest. Slides were incubated 1 hr at 90°C and G- and C-banded and AgNOR-stained as described by vanTuinen and Ledbetter (1983).

Meiotic chromosome preparation

Testicular (wedge) biopsies were obtained from two sibling male *H. agilis* (HAA402 and HAA404) heterozygous for both a double pericentric-inversion and a reciprocal-translocation. Specimens were anesthetized with 0.01 ml acepromazine maleate and 0.5 ml ketamine hydrochloride injected intramuscularly. Testicular material was processed both for pachytene (synaptonemal-complex, or SC) and diakinesis/metaphase I chromosomal analyses. Synaptonemal-complex preparations followed the modified surface-spreading technique of Sudman (1989). These surface-spread preparations were stained with silver nitrate (Howell and Black, 1980), mounted on 100-mesh copper grids, and examined using a JEM-100CX II transmission electron microscope at an accelerating voltage of 80 kV. Diakinesis/metaphase I preparations followed the technique of Sudman and Greenbaum (1989) and were either C-banded as described by Sumner (1972) or nondifferentially stained with Giemsa.

RESULTS

Inversions

Among the 63 animals studied, all three inversion morphs described by Stanyon et al. (1987) were observed (Figs. 1 and 2).

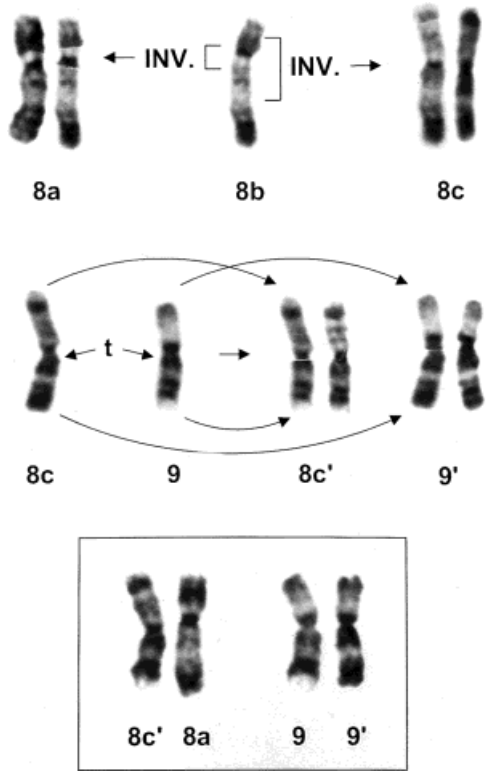

Fig. 2. G-banded photomicrographs of chromosomes 8 and 9 were artificially "rearranged" with scissors to show the progression of steps resulting in the complex polymorphic system in *H. agilis.* Independent artificial inversions of "8b" are shown alongside actual morphs "8a" and "8c" (first and fifth chromosomes of this group). Morph "8c" (bottom, left) is then involved in a translocation with chromosome 9. Artificial products are shown alongside actual morphs 8c′ and 9′ taken from *H. agilis* (the right members of each pair). The inset shows pairs 8 and 9 taken from a compoundly heterozygous specimen (8ac′), illustrating the difficulty in reconciling these two pairs without knowledge of the intermediates.

Their designations "8a," "8b," and "8c" are followed here. Our estimation of the breakpoints leading to the two presumptive derivative forms (Fig. 2) does not differ markedly from that of Stanyon et al. (1987) in that (1) both inversions are pericentric and (2) the breakpoints resulting in "c" are distal to those resulting in "a," i.e., "a" is a nested inversion. An exceedingly small pericentric-inversion postulated by Stanyon et al. (1987) was not confirmed. However, substantial amounts of centromeric heterochromatin occur on this chromosome pair (Tantravahi et al., 1975), and variation therein might not be distinguishable from subtle structural variation.

All three inversion morphs were observed in *H. lar, H. muelleri,* and *H. agilis,* but not in *H. pileatus,* which exhibits only the "b" morph, or *H. moloch,* which did not show the "a" morph (Table 1). Consolidation of our data with those of all previous interpretable studies (Table 2) indicated that all three morphs have been observed in *H. muelleri, H. agilis, H. moloch,* and *H. lar,* although the "a" morph was observed in only three of 26 specimens of *H. lar.* A single *H. klossii* (Kloss's gibbon), the only $2n = 44$ species not karyotyped here, was homozygous "bb." The pooled data for *H. pileatus* show that only the "b" morph has been observed.

Reciprocal translocation

In 16 of 18 specimens of *H. agilis* one or both chromosomes 8 were represented by a metacentric chromosome that resembles morph "c," but which, compared to the other species, differs in the length and banding pattern of the q-arm. Distal 8q in these specimens exhibited only two major dark G-bands instead of the three characteristic of chromosome 8 in other gibbons. In these specimens, one or both chromosomes 9 also had altered appearance compared to this pair in other species studied: 9q was longer and exhibited three distal dark bands instead of two. Comparison of G-banded metaphase chromosomes suggests the occurrence of a reciprocal-translocation involving chromosome 9 and morph "c" of chromosome 8, with breakpoints at or just below the centromeres of both chromosomes (Figs. 2 and 3). The translocated 8 is designated "8c′." ("c′" denotes that the reciprocal-translocation product "9c′" is present as well.) The translocation breakpoint on chromosome 8 was apparently within the region bounded by both inversions.

Table 1 shows that 24 of 36 chromosomes 8 in our *H. agilis* were "c′," making it the most common morph. All 16 specimens bearing "c′" morphs had balanced chromosomal complements, as judged by the cytogenetic

TABLE 1. Occurrence of chromosome 8 and 9 morphs in 2n = 44 species of Hylobates

Species, subspecies	Sex	Facility	ID#	Morphs[2]	Capt[1]/wild
H. pileatus	M	ICGS[3]	HP101	bb	wild
H. pileatus	M	ICGS	HP112	bb	wild
H. pileatus	M	ICGS	HP114	bb	wild
H. pileatus	F	ICGS	HP115	bb	wild
H. pileatus	M	ICGS	HP116	bb	capt
H. pileatus	F	ICGS	HP117	bb	capt
H. pileatus	M	ICGS	HP118	bb	capt
H. pileatus	F	ICGS	HP119	bb	wild
H. pileatus	F	Brownsville, TX	102	bb	unk
H. pileatus	F	Brownsville	105	bb	unk
H. lar	M	ICGS	HL004	bb	capt
H. lar	F	ICGS	HL041	bb	capt
H. lar	F	ICGS	HL055	bb	capt
H. lar	F	ICGS	HL059	bb	capt
H. lar	F	Lodi, CA	000081	bb	capt
H. lar	M	San Antonio, TX	540001	bb	wild
H. lar	F	San Antonio	540002	bb	wild
H. lar	M	Fort Worth, TX	373	bb	unk
H. lar	F	Fort Worth	433	bb	capt
H. lar	F	Fort Worth	669	bb	capt
H. lar	F	Dallas, TX	001194	ac	capt
H. lar	M	Dallas	823238	bb	wild
H. lar	M	Dallas	864781	bc	capt
H. lar	F	Dallas	896664	ab	capt
H. lar	M	Utica, NY	187042	bb	capt
H. lar	F	Brownsville	1272	bc	unk
H. lar	F	Brownsville	3843	bb	capt
H. lar	F	Brownsville	5276	bc	capt
H. muelleri abbotti	M	Brownsville	3135	aa	capt
H. m. funereus	F	San Antonio	690701	aa	wild
H. m. funereus	F	San Antonio	690702	aa	wild
H. m. funereus	F	ICGS	HMF505	bc	wild
H. m. funereus	M	San Antonio	870689	ac	wild
H. moloch	F	ICGS	HMO801	bb	wild
H. moloch	M	ICGS	HMO802	bb	wild
H. moloch	F	ICGS	HMO803	cc	capt
H. moloch	M	ICGS	HMO804	bb	capt
H. moloch	M	ICGS	HMO806	bc	capt
H. moloch	M	ICGS	HMO808	bb	wild
H. moloch	M	Perth, Austral.	80036	bb	capt
H. agilis agilis	M	Madison, WI	521A	ac′	wild
H. a. agilis	F	Houston, TX	690	c′c′	wild
H. a. agilis	F	ICGS	HAA401	c′c′	wild
H. a. agilis	M	ICGS	HAA402	ac′	capt
H. a. agilis	F	ICGS	HAA403	c′c′	wild
H. a. agilis	M	ICGS	HAA404	ac′	capt
H. a. agilis	M	ICGS	HAA406	c′c′	capt
H. a. agilis	F	ICGS	HAA445	c′c′	wild
H. a. agilis	M	ICGS	HAA496	c′c′	capt
H. a. agilis	M	ICGS	HAA498	c′c′	capt
H. a. agilis	M	ICGS	HAA499	ac′	capt
H. a. unko	F	ICGS	HAU405	ac′	capt
H. a. unko	M	ICGS	HAU408	ac′	wild
H. a. unko	M	ICGS	HAU410	c′c′	capt
H. a. unko	M	ICGS	HAU412	ac′	capt
H. a. albibarbis	F	Monroe, LA	112105	ac	wild
H. a. albibarbis	M	San Diego, CA	588335	bc′	capt
H. a. albibarbis	F	San Diego	588336	cc	wild
Hybrids					
H. m. funereus (690701) × *H. a. agilis* (721911)	F	San Antonio, TX	790584	ac′	capt
	F	San Antonio	830310	ac′	capt
	F	San Antonio	851085	ac′	capt
H. m. funereus (112104) × *H. a. albibarbis* (112105)	M	Plumpton, MD	M6	ac	capt
H. lar × *H. a. agilis*	M	Phoenix, AZ	none	ab	capt

[1] "Capt" denotes captive born; "unk" denotes unknown.
[2] The "c′" morph denotes the chromosome 8 and the chromosome 9 translocation products.
[3] International Center for Gibbon Studies, Santa Clarita, CA.

TABLE 2. Pooled inversion and translocation morph frequencies in 2n = 44 gibbons[1,2]

Species	a	b	c	c′
H. klossii		2		
H. pileatus	—	22	—	—
H. lar	3	43	6	—
H. muelleri	9	2	9	—
H. moloch	3	13	6	—
H. agilis	9	3	8	24

[1] Based on examination of published karyotypes and data from the following sources: Warburton et al. (1975), Tantravahi et al. (1975), Dutrillaux et al. (1975), Hsu and Benirschke (1977), Myers and Shafer (1979), vanTuinen and Ledbetter (1983), Stanyon et al. (1987), and the present study. Animals common to more than one study are represented only once.

[2] This tabulation takes into account correction of previous misidentification of the following animals: "*H. moloch*" of Tantravahi et al. (1975) is *H. muelleri abbotti.* From Stanyon et al. (1987): three "*H. muelleri*" (nos. 6710, 735, and 737) are *H. lar* (B. Swenson, personal communication). Only four of their eight *H. agilis* (nos. 3, 5, 7, and 10) are included here since photos of only these four were available to us for species identification. The following previously typed chromosomes were corrected: *H. muelleri abbotti* submetacentric 8 in Tantravahi et al. (1975) is "b," not "a" as stated by Stanyon et al. (1987); *H. agilis* submetactric 8 in vanTuinen and Ledbetter (1983) is "a," not "b" as stated by Stanyon et al. (1987).

resolution attained. The sire and dam of three *H. muelleri* × *H. agilis* hybrids were a *H. agilis* male 721911 (not karyotyped) and *H. muelleri* female 690702, who was "aa" (Table 1). Because the hybrids were all "c′," the sire carried at least one "c′."

Meiosis

To confirm the existence of translocation polymorphism inferred from G-banded somatic cells, we analyzed meiotic cells from two male *H. agilis* scored as heterozygous for the reciprocal-translocation, and which were heterozygous for both inversions as well (i.e., they were "ac′"). This provided an opportunity to observe the complex configurations that might result from the simultaneous presence of these rearrangements. It should be noted that morphs "a" and "c" differed by two inversion steps, and therefore "a" and "c′" differed by a large inversion, a second inversion that would behave as a nested inversion in meiosis, and a reciprocal-translocation with a breakpoint within the nested inversion (Figs. 2 and 3).

In homozygous individuals diakinesis/metaphase I cells are expected to have 22 bivalents, and this was so noted by Chiarelli (1972). In our males, clearly delineated cells had 20 bivalents and one quadrivalent, confirming the exchange of material concomitant with reciprocal-translocation. All 36 diakinesis/metaphase I cells analyzed displayed a ring quadrivalent configuration (Fig. 4). In many of these quadrivalents, four clearly observable centromeres were seen. Nonterminalized chiasmata were often observed within the quadrivalent (Fig. 4). Within the X-Y bivalent, the minute Y chromosome was usually associated with the distal end of the submetacentric X chromosome.

Electron-microscopic analysis of SC configurations facilitated more detailed visualization of the extent and fidelity of homologous synapsis at pachynema. In comparison to the theoretically complex topology of pachytene quadrivalents based on maximal homologous pairing (Fig. 5), the observed SCs were relatively simple. The predominant type of pachytene configuration observed for the inversion-translocation quadrivalent was consistent with that expected of translocation heterozygotes (Fig. 6). Synapsis was apparently initiated at, and proceeded from, all four pairs of telomeres, as homologous pairing was observed in all four arms distal to the inverted region. However, inversion loops that would reflect complete homologous pairing (homosynapsis) were absent (Fig. 6a). The "H"-shape of most pachytene configurations (Fig. 6c) was consistent with nonhomologous pairing (heterosynapsis) of the inverted segments. Other quadrivalents exhibited asynapsis in the inverted region, occasionally with thickening and apparent shortening of the axis of the putative chromosome 8 non-translocated "a" morph.

While analysis of banded metaphase chromosomes suggests that the large inversion encompassed about 50% of the length of chromosome 8, the unpaired segments in SCs typically occupied much less than 50% of the chromosome length. This implied differential contraction of the inverted region, and/or progression of synapsis from the arms leading to heterosynapsis in the inversion region.

In pachytene nuclei the X-Y bivalent was readily distinguishable by its dense staining and heteromorphic appearance. Notably, as-

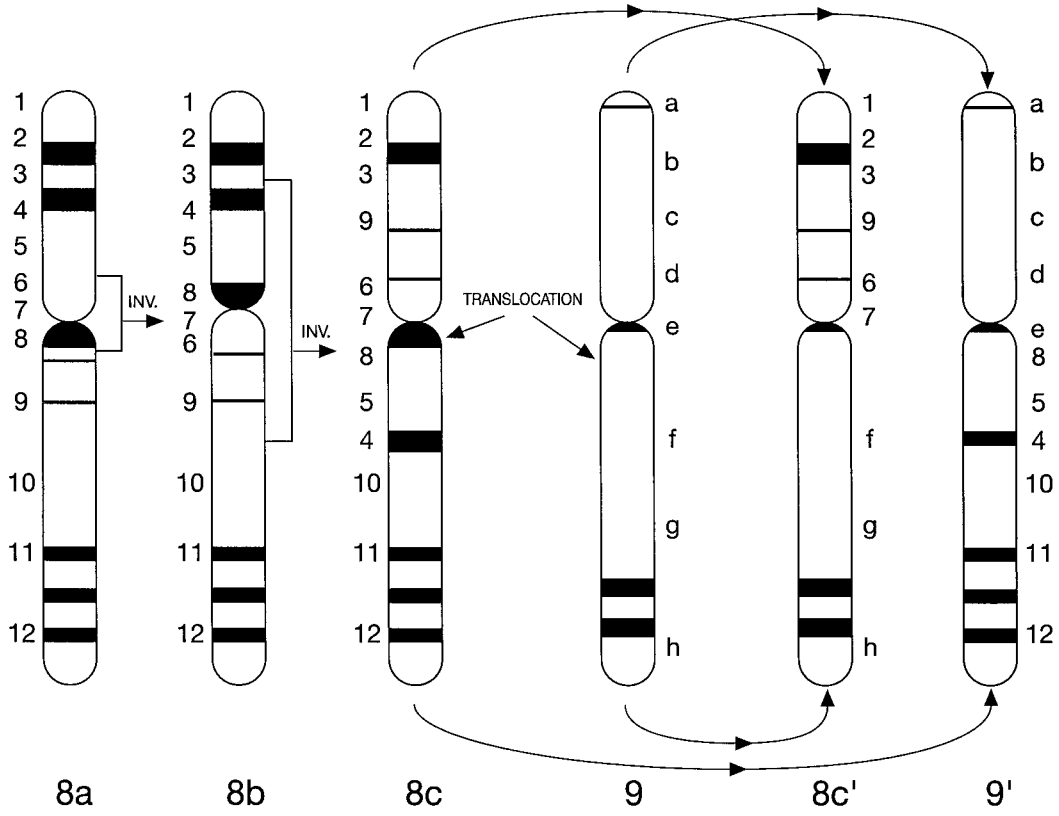

Fig. 3. Diagrammatic representation of series of rearrangements yielding the chromosome 8 and 9 morphs observed in extant specimens. The left-to-right order of changes is presented as such in order to most simply contrast the morphs "8a" and "8c′." (Note: If "8b" was the ancestral configuration the arrow from "8a" to "8b" would be reversed, see text). Looped arrows show the fate of the four chromosome regions involved in the reciprocal translocation. Letters and numbers illustrate the reordering of hypothetical genomic regions relative to the configurations "8a" and "9."

sociation of the quadrivalents with the sex bivalent was observed in only one nucleus.

DISCUSSION

Compound chromosomal polymorphisms

An earlier study involving broad comparisons between different subgenera of *Hylobates* included a male and a female *H. agilis* (specimens 521A and 690, respectively, Table 1). Only tentative conclusions could be drawn concerning the male, which had an inversion heteromorphism involving pair 8 (vanTuinen and Ledbetter, 1983). This karyotype was further complicated by an inability to visually match both members of pair 9 (which we now know is also heteromorphic) and distinguish one of them from pair 15. The discrepancies could not be reconciled by any obvious intermediate rearrangement, and it was speculated that "at least two independent rearrangements are represented in this animal" (vanTuinen and Ledbetter, 1983, p. 459). We have since determined that pair 15 was heteromorphic in the amount of centromeric heterochromatin. With the ability to now fully interpret these earlier findings, we recognize in this male, as in the two male compound heterozygotes described in the present study, that among pairs 8 and 9, no two chromosomes appeared fully homologous (Fig. 2), and in fact *three* rearrangements are represented in these animals.

Since the earlier study, we have obtained specimens revealing all intermediate stage chromosomes bridging these two pairs, and

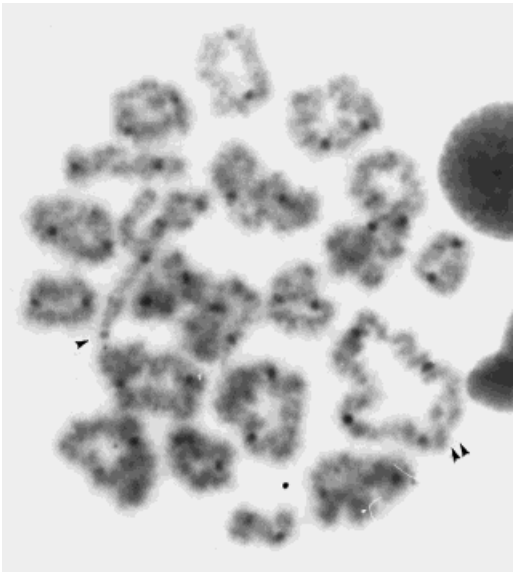

Fig. 4. Diakinesis/metaphase I cell from testicular biopsy of inversion-translocation heterozygote. Twenty bivalents including the X-Y bivalent are observed. The small Y chromosome (arrowhead) is associated with the distal long arm of the X. A single ring quadrivalent (double arrowheads) shows four distinct centromeres, and depicts the most common configuration for this element in diakinesis/metaphase I.

these were necessary to resolve the complex polymorphisms. A minimum of two heterozygous animals with four different chromosome 8 morphs are necessary to reconstruct the steps. In addition, in the present study, specimens from related species lacking the translocation polymorphism were compared to establish a likely ancestral condition and sequence of changes. This ancestral configuration may have been "8b," since it is the only morph present in all taxa studied. Alternatively, all three inversion morphs may have existed in all of these taxa following speciation, with "a" and "c" being lost during divergence of *H. pileatus,* leaving the ancestral state open to question. Whatever the direction of change, we now know from comparative G-banding and meiotic studies that chromosome pairs 8 and 9 in *H. agilis* actually encompass three independent rearrangements, a reciprocal-translocation and two pericentric-inversions, from an ancestral configuration. In compound heterozygotes, the theoretical meiotic pairing configuration features a nested inversion loop and a reciprocal-translocation quadrivalent (Fig. 5). To our knowledge this complex, compound combination of polymorphisms has not been described in other mammals. It should be noted that in all, there are 10 distinct karyotypes that constitute the various possible balanced combinations of chromosome 8 and 9 morphs: aa, ab, ac, bb, bc, cc, ac′, bc′, cc′, and c′c′. Of these 10, three (ab, bc, and cc′) have not yet been reported in *H. agilis.*

Fertility in heteromorphic gibbons

That inversion and translocation polymorphism occur in hylobatids is not unexpected. Previous comparative cytogenetic and gene mapping studies suggest rapid and extensive genomic remodeling by both mechanisms, and intermediate polymorphic states are presumed to precede fixation of new morphs. However, the occurrence of multiple inversion morphs coupled with a translocation, all within a single restricted chromosomal region, raises questions concerning fertility of heterozygotes. Inspection of Table 1 shows that neither inversion heterozygotes nor inversion/translocation compound heterozygotes are rare: 8 of 18 *H. agilis* are compound heterozygotes, one is an inversion heterozygote and nine are homozygous. Therefore the versatility of the meiotic apparatus will be frequently challenged in *H. agilis.*

Structural heterozygosity in gibbons suggests several questions concerning their fertility: (1) Does inversion polymorphism constitute a recombinational genetic load for the species by lowering fertility of heterozygotes (of which there are three types); (2) similarly, do simple translocation heterozygotes harbor a genetic load; and (3) do inversion and translocation meiotic configurations interact or interfere in such a way as to either enhance or hamper the segregation of balanced chromosome complements in compound heterozygotes? Males with karyotypes described in points (1) and (2) were not available for study. However, two males compoundly heterozygous for the double-inversion and reciprocal-translocation were available, and meiotic phenomena associated with compound heterozygosity were examined. Two measures of reproductive

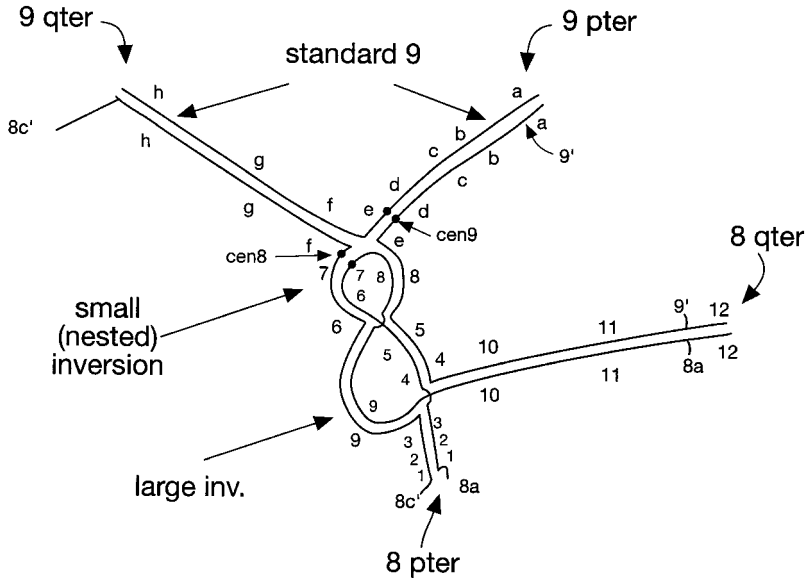

Fig. 5. Theoretical configuration of double inversion-translocation heterozygote in meiosis, assuming complete homologous pairing throughout the quadrivalent. Two inversion loops are shown (the smaller one nested) as well as the four elements resulting from translocation. Relative sizes of the various segments are estimated from G-banded chromosomes and are approximate. Letters and numbers denote the same hypothetical genomic regions employed in Figure 3. cen = centromere; ter = terminus.

fitness, analysis of meiotic configurations and evaluation of offspring, can be considered.

Meiotic assessment. Data gathered here suggest little if any diminution of fertility in heterozygous gibbons. First, the substantial length of the translocated segments (approximately 50% of the length of each chromosome) should promote crossing over. Indeed, diakinesis/metaphase I configurations (Fig. 4) consistently showed ring quadrivalent formation, indicating that chiasmata regularly form between homosynapsed regions in all four arms. This should favor segregation of the four chromosomes in a proper disjunctional manner (White, 1973).

Synaptonemal-complex configurations consistently show synapsis in the four arms, further suggesting an extent of pairing adequate for chiasma formation (Fig. 6). Second, the proximity of the translocation breakpoints to the centromere is important, as the interstitial (centromere to breakpoint) segments are extremely short (Fig. 2). Consequently, crossovers within the interstitial regions should be very unlikely, thereby favoring alternate (balanced) over adjacent segregation (White, 1973). Third, whereas double inversion loops comprising 50% of the chromosomal length would plausibly allow intra-loop crossover (resulting in duplication-deficiency gametes) inversion loops were never observed in SCs of the two male *H. agilis.* During pachynema, the inverted segments progress directly to heterosynapsis without prior homosynapsis, a pattern similar to that noted in inversion heterozygotes in the sand rat *Psammomys obesus,* whereby asynapsis of the inverted region extended into early pachynema (Ashley et al., 1981). The observed heterosynapsis was similar to that seen in deer mouse *Peromyscus* inversion heterozygotes (Hale, 1986). In sand rats, deer mice, and apparently gibbons, heterosynapsis of the inverted segments precludes crossing over, thereby averting the production of duplication-deficiency gametes. In summary, all cytogenetic data are consistent with normal disjunction of homologous regions and balanced gametic complements in structural heterozygotes. The absence of inversion loops may be attrib-

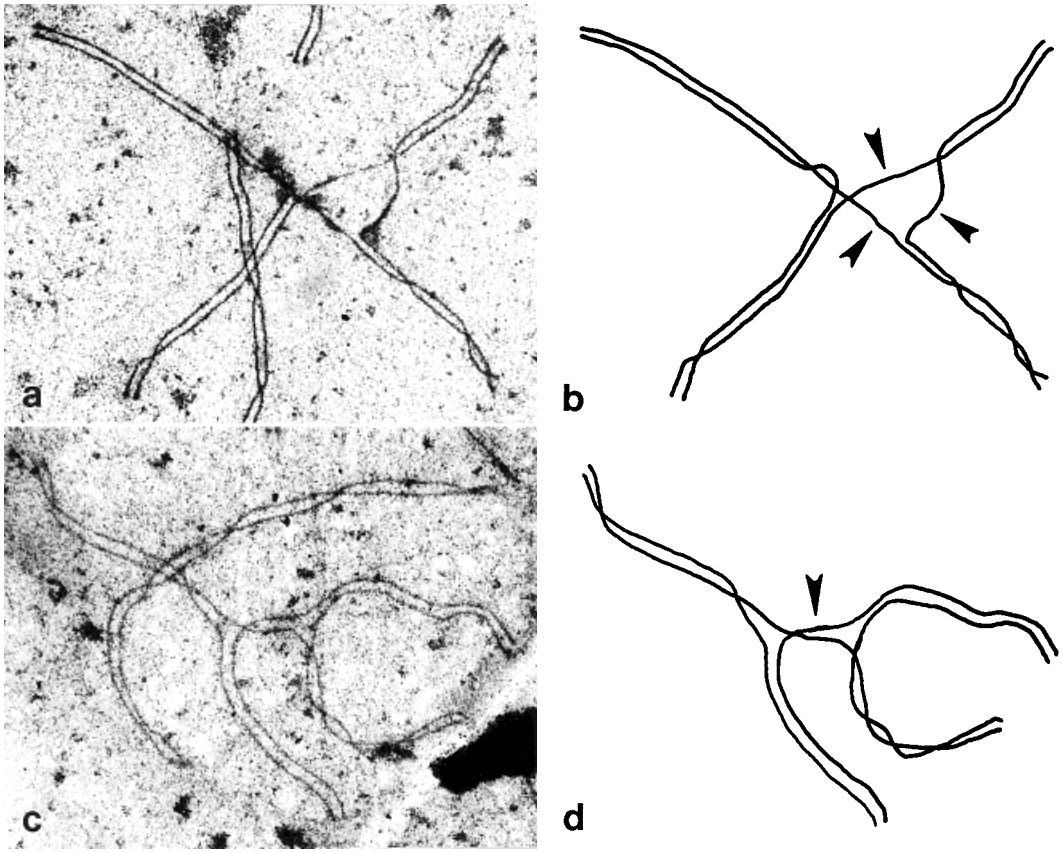

Fig. 6. Electron micrographs of inversion-translocation quadrivalents showing chromosome pairs 8 and 9 in pachytene (left), with diagrammatic representation of photomicrographs (right of each pair). (**a**) Partially synapsed translocation quadrivalent in an early-pachytene nucleus. Synapsis has been initiated from all four telomeres, and the paired segments are presumably homosynapsed. An interstitial region remains asynapsed. Original magnification: 8,275×. (**b**) Arrowheads indicate the unpaired region. The synaptonemal complex overlying the quadrivalent in (a) has been omitted. (**c**) Fully synapsed translocation quadrivalent in a mid-pachytene nucleus. The "H"-shape of this configuration is indicative of heterosynapsis of segments involving the double inversion, and is typical of the majority of the translocation quadrivalents observed in pachytene nuclei. Original magnification 10,440×. (**d**) Arrowhead indicates a heterosynapsed region within the quadrivalent. The synaptonemal complex overlying the fully synapsed quadrivalent in (c) has been omitted.

uted to any of several mechanisms. Although the inverted region is sufficiently large to favor loop formation, the topological complexity of the heteromorphisms described here may exceed the ability of the meiotic apparatus to achieve homologous pairing in a compound inverted loop. Alternatively, the lack of homology may escape recognition due to apposition of G-light and G-dark bands during meiotic pairing, an hypothesis advanced by Ashley (1988) and dePerdigo et al. (1989). Inspection of G-band patterns of the inverted regions indicates that indeed this condition would result following heterosynapsis, thereby avoiding a transition to homologous pairing and the deleterious consequences of crossing over.

Finally, in the inverted region there may be an absence of "synaptic initiation loci," hypothetical sites which are postulated to be points of origin for synapsis (Moses et al., 1982). Homosynapsis would be contingent on activity of at least three synaptic loci, two flanking and one within the inverted region.

The asynapsed regions of the ring quadrivalent apparently do not associate with the X-Y bivalent. Sterility in structural heterozygotes of both humans and laboratory mice

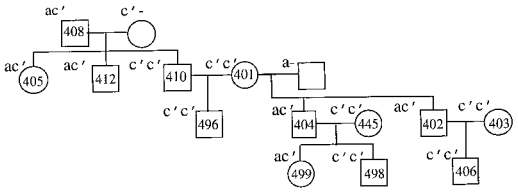

Fig. 7. *H. agilis* pedigree showing inheritance of chromosome 8 and 9 morphs and viable offspring of heterozygous "c′" carriers. Karyotypes of two individuals not studied were partly reconstructed from the offspring karyotypes.

has been attributed to association of unpaired autosomal regions with asynapsed regions of the X and Y chromosomes (Forejt, 1982; Johannisson et al., 1987). The genetic basis of the sterility is hypothesized to be caused by interference with normal inactivation of X-linked loci. The apparent lack of association between the X-Y bivalent and asynapsed quadrivalents in *H. agilis* is consistent with unencumbered meiotic function.

Reproductive success. Direct evidence for fertility is the production of normal offspring by the compound heterozygotes we examined. Male HAA402 sired male offspring HAA406 by female HAA403, male HAA404 sired a female HAA499 and a male HAA498 by female HAA445, and male HAA408 sired males HAA410 and HAA412 and female HAA405 by a female not studied (Fig. 7). These offspring have normal phenotypes and balanced karyotypes. In addition, *H. agilis* 112105, a double inversion heterozygote of the form "ac," mated with *H. muelleri* 112104 (not karyotyped), producing hybrid offspring M6 (Table 1). Karyotypic analysis of the hybrid individual revealed a balanced complement of the form "ac." Therefore if structural heterozygosity is curtailing fertility in *H. agilis,* the effects may be inconsequential, as they are not apparent from these data.

Among other hominoid primates, the most complex polymorphisms are found in Bornean and Sumatran orangutans (*Pongo pygmaeus*), which are polymorphic for an inverted insertion (three break) rearrangement (Turleau et al., 1975). At least one heterozygous individual has proven fertility (Turleau et al., 1975), and gross sperm morphology in heterozygotes appears to be normal (Seuanez et al., 1976). It has been speculated that crossing-over in inversion heterozygotes occurs outside of inversion loops (Seuanez et al., 1976).

Captive gibbon management

Two issues relevant to the management of captive hylobatids can be addressed in light of the polymorphisms described here. In other mammalian species, it is of paramount importance to avoid captive breeding strategies that result in semi-fertile or sterile chromosomally heterozygous animals. Unfortunate examples of offspring produced by inadvertent or unwitting hybridization of chromosomally disparate animals have been discussed by Benirschke and Kumamoto (1991). By contrast, the present study suggests that chromosomal heterogeneity does not compromise the propogation of 44-chromosome gibbons. Indeed, the ease with which these taxa can interbreed and bear fertile offspring is the greater problem in gibbon management, as this erodes species' distinctions and undermines maintenance of pure populations. The fertility of hybrids between 44-chromosome species is well established, to wit a hybrid offspring resulting from a captive mating now known to be *H. lar* × *H. agilis* had born F2 offspring (A.R. Mootnick and L.A. Prouty, unpublished data).

The ability of gibbon species to hybridize is pertinent to the second management issue, i.e., the power of cytogenetic analysis in correctly identifying taxa prior to establishment of breeding regimes. At the present time the data suggest two useful applications of cytogenetic analysis in the captive management of gibbons. First, pooled data (Table 2) strongly suggest that *H. pileatus* possesses only the "b" morph of chromosome 8, as all eleven specimens karyotyped thus far are "bb." If this inference is corroborated by further study, then the designation *H. pileatus* can be excluded in specimens carrying "a," "c," or "c′" morphs. (This may be more pertinent to identification of putative hybrids since pure *H. pileatus* specimens are phenotypically distinctive.)

Second, the existence of a translocation possibly unique to *H. agilis* allows positive identification of perhaps as many as 85–90% of such specimens (the figure derived from Hardy-Weinberg calculations of the "c′" morph frequency in our specimens). Frequent misidentification of *H. agilis, H. moloch,* and *H. muelleri,* has been encountered in the literature (see Table 2 legend), and has been revealed through correspondence with zoological institutions. For instance, a *H. agilis* male 588335 was imported as a *H. moloch* and was finally correctly assigned by one of us (ARM) following phenotypic analyses. This male exhibited the distinctive "c′" morph and therefore could have been correctly identified by karyotypic analysis. Furthermore, Hardy-Weinberg calculations suggest that approximately 60–65% of F1 hybrids of *H. agilis* could be identified as having *H. agilis* parentage. For instance, in one of three interspecies matings involving *H. agilis* (Table 1), the distinctive "c′" morph was seen in three hybrid offspring, revealing the male (721911, not karyoyped) to be an obligate carrier of the "c′" morph and reflecting the sire's known assignation of *H. agilis* (the *H. muelleri* dam was misclassified in this instance). Conversely, the *H. agilis* dam 112105 of a second mating was "ac" (studied retrospectively), consequently she is not informative. Similarly, the offspring of the third hybrid cross *H. lar* × *H. agilis agilis* was "ab." These latter two examples represent the calculated 35–40% of *H. agilis* hybrid offspring that will lack the "c′" marker, illustrating the limitation imposed by the fact that the unique "c′" morph has not replaced the other morphs in *H. agilis.* Nevertheless, the ability to detect misidentification of *H. agilis* is now greatly improved. Substantiation of this association is contingent on the analysis of additional specimens with special attention to chromosomes 8 and 9, especially in *H. klossii,* of which so few specimens have been studied to date.

An intriguing but preliminary finding may lend support to taxonomic revision in *H. moloch,* with implications for management. Recent findings from pelage, vocalization, mitochondrial DNA analysis, and geographic distribution suggest that it may constitute two subspecies (J. Supriatna, personal communication). Of our six specimens, only HMO803 and HMO806 carry morph "c"; these two also have deeper pitched vocalization than the other five specimens. Only further phenotypic, cytogenetic, and molecular studies can strengthen the suggestion that this apparent correspondence reflects subspecies distinction.

The relevance of chromosome studies in sustaining primate populations has been emphasized by Seuanez (1986). Cytogenetic analysis has been applied to the management of *Aotus* (owl monkey) (Elliott et al., 1976), *Ateles* (spider monkey) (deBoer and deBruijn, 1990), *Saimiri* (squirrel monkey) (Fogle, 1990), and orangutan (Seuanez, 1982). Couturier and Lernould (1991) reported karyotypic distinction between four taxa in the hylobatid subgenus *Nomascus,* all 2n = 52 but differing by inversion and/or reciprocal-translocation. The suggestion of Benirschke and Kumamoto (1991) to establish karyotypes for taxa, then apply cytogenetic analysis prior to mating animals, is germane to the 44-chromosome gibbons. The application of cytogenetic studies in conservation of this threatened primate family is thus expanding and has considerable practical, not merely scientific, merit.

ACKNOWLEDGMENTS

The following institutions generously supported this study by providing specimens from their animals: Houston Zoological Gardens, Houston, TX; Dallas Zoo, Dallas, TX; Ft. Worth Zoological Park, Ft. Worth, TX; Gladys Porter Zoo, Brownsville, TX; Henry Vilas Zoo, Madison, WI; Louisiana Purchase Gardens and Zoo, Monroe, LA; Micke Grove Zoo, Lodi, CA; San Antonio Zoological Gardens and Aquarium, San Antonio, TX; San Diego Zoo, San Diego, CA; and Utica Zoo, Utica, NY. Dr. Marie Mulcahy of the State Health/Clinical Cytogenetics Service, Perth, Western Australia, provided karyotypes on a *H. moloch* from Perth Zoological Gardens. We gratefully acknowledge Max McElroy, D.V.M. for veterinary assistance, Dr. David Chivers for confirming the vocalization of the male *H. muelleri abbotti,* Dr. Joe Marshall for confirming the vocalization of two groups of *H. lar,* and Terry Olsen for technical illustrations. D.W.H. was supported by

National Science Foundation grant DEB-9201509.

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